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L3: Entry 1 of 1 File: USPT Mar 26, 1996

DOCUMENT-IDENTIFIER: US 5501959 A
TITLE: Antibiotic and cytotoxic drug susceptibility assays using resazurin and poising agents

Detailed Description Text (29):
Since resazurin is non-fluorescing and resorufin is strongly fluorescing at a wavelength of 580 nanometers, the test panel 100 may be read using a fluorometer in accordance with a fluorescence excitation reading protocol using a reading system illustrated schematically in FIG. 29. An excitation source having a wavelength at 560 or below is used to excite fluorescence emission from resorufin in the wells. Adequate separation of excitation and emission wavelengths should be maintained. Each test well 101-103 of the test panel 100 is scanned with respect to the exciting light source and detector so that the value of fluorescence excitation of resorufin in each of the three wells is obtained. For purposes of this explanation, the value obtained from the negative growth control well is designated N, the value from the positive growth control well is designated P, and the value from the test well is designated T. This reading of the panel is done after incubation of the test panel has been done for a period of time sufficient to cause a substantial production of resorufin in wells in which organism growth is occurring, usually at least 3 hours and preferring 5 hours, or more with a particularly useful protocol relying on overnight incubation of the cellular sample. The values of N and P are then examined to determine if the data correspond with a valid test. The value of N is compared with, and for valid panel data must be below, a threshold value Nf which has been determined to be indicative of a failed test due to the presence of too much resorufin in the negative growth control well due to contamination of the panel or some other cause. The value of P is compared with, and for valid panel data must be above, a threshold value Pf which has been determined to be indicative of a failed test due to the presence of too little resorufin in the positive growth control well after the incubation period due to a failure of the growth medium to promote organism growth or other causes. If the panel data passes these validation tests, then the test well data can be operated on in accordance with the rest of the fluorescence excitation reading protocol.

Detailed Description Text (91):
FIG. 29 shows that, in the case of visible light reflectance reading for implementing a visible light reading protocol, a single filter may be used for selection of the reading wavelength which will be used to determine the reflected color characteristics of the liquid in the test well. Multiple wavelength analysis could also be used if desired. FIG. 30 illustrates that, in the case of fluorescence excitation reading for implementing a fluorescence excitation reading protocol, separate filters are employed for selecting the exciting wavelength and the emission wavelength. Both visible light reading of reflected color and fluorescence reading can be implemented with instrumentation that is currently commercially available and known to persons familiar with this technology.

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